

Permanent translocation heterozygosity in dioecious *Baccharis coridifolia* DC. (Asteraceae)

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The karyotype of *Baccharis coridifolia* DC., a dioecious diploid species consists of 16 chromosomes with median (**m**) and 2 with submedian centromeric positions (**sm**) ($2n = 18$). Meiotic analyses of 219 male plants belonging to 12 populations showed across a wide geographical range one quadrivalent and seven bivalents in all of them. The quadrivalent involves the four biggest **m** chromosomes and probably constitutes a sex multivalent carrying in tight linkage the sex genes and provides a mechanism whereby permanent translocation heterozygosity is maintained. In metaphase I alternate orientation of the quadrivalent was predominant (85.71%). Fourteen megaspore mother cells were analyzed and did not show the quadrivalent. A model for the origin and maintenance of the sexual translocation system is presented. It is assumed that there are two non-allelic factors **M** and **n**; **M** would be a dominant female suppressor and **n** a recessive male-suppressor. The excess of male plants observed in several populations at different localities might result from heterotic effects of some gene complexes carried by the translocation. This excess probably ensures a good supply of pollen for the vagaries of insect pollination at flowering time.

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Baccharis (Asteraceae) is a New World genus of approximately 450 dioecious species. About 100 species are diploids with 18 chromosomes and a few are polyploids according to present chromosome indexes.

Some species are weeds, or are toxic. Within the latter group, *B. coridifolia* DC., known as “romerillo” or “mío-mío”, is highly toxic for cattle, horses and sheep in all its area of distribution (Brazil, Bolivia, Paraguay, Uruguay, north and center of Argentina) (RAGONESE and MILANO 1984).

HUNZIKER et al. (1990) observed the presence of seven bivalents plus one quadrivalent in all 15 cells of one male plant from Tandil (Buenos Aires Province, Argentina). They concluded that the individual plant was heterozygous for a reciprocal translocation. HUNZIKER and WULFF (1990) reported the presence of more individuals heterozygous for the reciprocal translocation from Tandil and WULFF and HUNZIKER (1995) reported summarily 184 male plants from 10 localities and found also that all were structural heterozygotes.

In order to determine the frequency and geographic distribution of the translocation and the nature of the mechanism that maintains the structural heterozygosity, 35 male and eleven additional female individuals belonging to 12 populations from Bolivia, Brazil and Argentina have now been cytologically analyzed and all data are reported in detail in the present contribution.

MATERIALS AND METHODS

Provenance, number of individuals and collectors of *B. coridifolia* are given in Table 1. The geographical distribution of the sampled populations is shown in Fig. 1. Voucher specimens for each sample are deposited at the following herbaria: Instituto de Botánica Darwinion (SI) and Facultad de Ciencias Exactas y Naturales, Buenos Aires (BAFC). The abbreviations for the herbaria follow HOLMGREN et al. (1990).

Mature cypselae were germinated at $20^{\circ} \pm 2^{\circ}\text{C}$. Individual seed samples were sown no more than three months after the date of collection. Root tips were pre-treated with cold (0°C for 24 h), fixed in 3:1 (absolute ethanol: glacial acetic acid) and kept for 24 h at 4°C . Afterwards they were hydrolyzed in HCl 5N at 20°C for 15–20 min. Meiotic studies were performed on micro- or megaspore mother cells fixed in 6:3:1 (absolute ethanol: chloroform: glacial acetic acid). Slides were prepared by squashing root tips or dissecting anthers and ovules in iron-propionic haematoxylin (SÁEZ 1960; NÚÑEZ 1968). Permanent slides were obtained by the dry-ice method or by BRADLEY's (1948) method, in both cases dehydrating the slides in absolute alcohol and mounting in Euparal. Chromosome measurements for karyotypes are based on 10 karyograms using a Kontron Mini-mop image analyzer. Chromosomes were classified accord-

Table 1. *Accessions and collectors of the specimens of B. coridifolia analyzed (MDB = M.D. Bertoni; SD = S. Dieguez; VD = V. Dudás; JHH = J.H. Hunziker; JCG = J.C. Gamero; AFW = A.F. Wulff). Locality numbers are indicated in Fig. 1. Localities are arranged from North to South*

| Locality no. in Fig. 1 | No. of plants analyzed | Total number of cells | Accessions |
|------------------------|------------------------|-----------------------|---|
| 1 | 5 | 30 | Bolivia, Dep.Santa Cruz, Prov. Valle Grande, 7 km from Samaipata, ± 1950 m altitude. March 3,19 94. JHH & AFW 13012,13013 (SI). |
| 2 | 42 | 86 | Argentina, Prov. Salta, Dep. Caldera, Cuesta del Gallinato, 1450 m altitude. January 27, 1994. JHH, JCG & VD 12853 (SI). |
| 3 | 2 | 16 | Argentina, Prov. Tucumán, Dep. Tafí, San Javier, 1000 m altitude. March 31, 1998. AFW 795 (BAFC) |
| 4 | 8 | 32 | Brasil, Dep. Río Grande do Sul, between Santa Maria and Rosario do Sul. April13, 1991. MDB w/n. (BAFC). |
| 5 | 7 | 36 | Argentina, Prov. Corrientes, Dep. Curuzú-Cuatiá, 60 km South of Curuzú-Cuatiá. April 4, 1991. SD w/n. (BAFC). |
| 6 | 5 | 16 | Argentina, Prov. Entre Ríos, Dep. Uruguay, Herrera, April 4, 1991. SD s/n. (BAFC). |
| 7 | 38 | 55 | Argentina, Prov. Entre Ríos, Dep. Gualeguaychú, junction routes 12 and 16. February 2, 1993. JHH & JCG 12495 (SI). |
| 8 | 48 | 192 | Argentina, Prov. Entre Ríos, Dep. Gualeguaychú, Perdices, 27 Km North of Ceibas. February 2, 1993. JHH & JCG 12492 (SI). |
| 9 | 20 | 92 | Argentina, Prov. Buenos Aires, Tandil, January 15-16 1989, JHH 11576 (SI); April 14, 1989, JHH & JCG 11689, 11690, 11694 (SI); January 15-16, 1990, AFW 419, 428, 435 (BAFC). |
| 10 | 17 | 36 | Argentina, Prov. Buenos Aires, Balcarce, Sierra La Barrosa. February 12, 1993. JHH & JCG 12499 (SI). |
| 11 | 12 | 30 | Argentina, Prov. Buenos Aires, Balcarce, Sierra El Volcán. February 13, 1993. JHH & JCG 12500 (SI). |
| 12 | 15 | 42 | Argentina, Prov. Buenos Aires, Balcarce, Sierra La Vigilancia. February 13, 1993. JHH & JCG 12502 (SI). |
| | 219 | 663 | |

ing to LEVAN et al. (1964) as **m** or **sm** according to their median or sub-median centromeric positions.

Pollen stainability was determined with differential staining with acid fuchsin, orange G and malachite green (ALEXANDER 1969); 500 grains per individual were counted.

RESULTS

The karyotype of *B. coridifolia* is formed by 16 **m** + 2 **sm** chromosomes (Fig. 2). The four chromosomes involved in the translocation are the two largest **m** chromosome pairs. In the ten analyzable cells the largest pair of chromosomes measured 7.91 ± 0.05 μm and the smallest 4.55 ± 0.22 . One pair has a secondary constriction near the end of its longest arm. The **sm** pair is the smallest.

The meiotic analysis revealed the presence of 7 bivalents plus one quadrivalent in 663 cells studied in the 219 male individuals analyzed (Table 1, Fig. 2).

The mean chiasma frequency in diakinesis varied between populations from 16.3 ± 0.15 to 17.9 ± 0.08 (Table 2), the number of rod bivalents varied between 0 and 2. Chiasmata were always located at terminal positions in all bivalents (Fig. 3a, b). Chromosomes at male diakinesis revealed that the largest pairs of the complement were involved in the translocation (Fig. 3a, b). The smallest pair (no. 9) was recognized in many cells (Fig. 3a, b).

In one population (Table 3) 133 metaphase I cells of 12 male plants were studied: 90.98 % and 9.02 % of the quadrivalents were rings and chains, respectively; 85.71 % of the quadrivalents showed alternate orientation (Fig. 3d), 11.28 % had adjacent orientation (Fig. 3e) and 3 % showed linear orientation; in the former the central chromosomes are not oriented to either pole and may give rise later to "false univalents" (Fig. 8 in RIEGER et al. 1976).

Our data are probably somewhat distorted because cells with rings and chains with adjacent orientation



Fig. 1. Geographic distribution of the *Baccharis coridifolia* populations that were studied cytologically. For the names of the numbered localities see Table 1.

are more easily detected than those with alternate orientation and their estimated frequencies probably are higher than the real ones. It is also possible that quadrivalents showing adjacent orientation may re-orient by a correction of their attachments to microtubules (HEJNOWICZ and FELDMAN 2000).

Over 1100 ovules were dissected from many female plants in order to obtain 14 analyzable megaspore mother cells. In the latter no quadrivalent was observed and in 6 the two big bivalents (that in microspore mother cells form quadrivalent) were distinguished (Fig. 4).

Pollen stainability in male plants was determined in plants other than the ones studied cytologically. The stainability in 14 plants from diverse localities ranged from 93 to 97.7%; only two plants had 42.7 and

55.9%, respectively. Sex ratios were studied in two populations and were found to be male predominant, sex ratios ranging from 1.83 to 3.21 (Table 4). At other localities male plants were also more frequent than female in proportions that apparently were within those limits.

DISCUSSION

Meiosis has been studied in 219 male plants from a total of 12 populations throughout most of the range of *Baccharis coridifolia* (Bolivia to central eastern Argentina). All staminate plants had in every meicyte studied one quadrivalent involving the four largest metacentric chromosomes, leaving no doubt that translocation heterozygosity in the male is per-

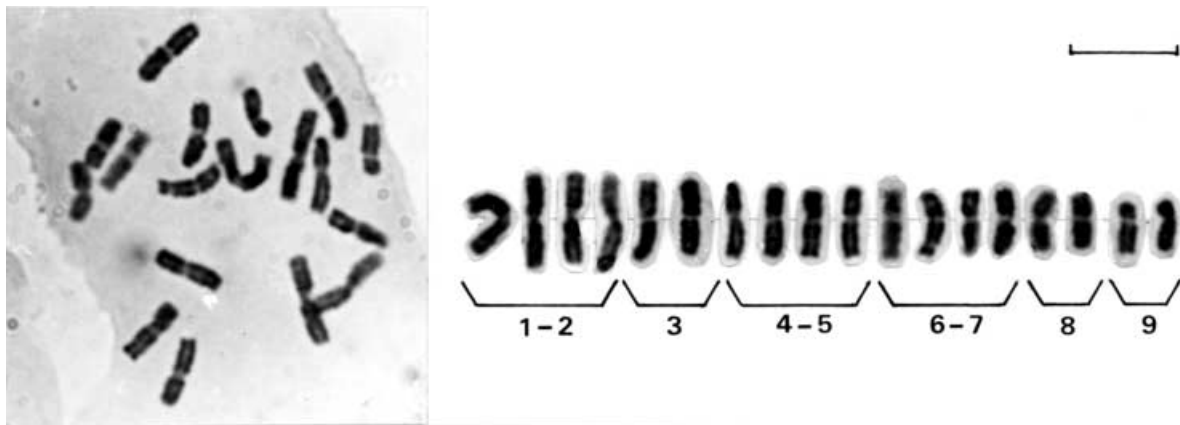


Fig. 2. Karyogram of *Baccharis coridifolia* and the cell used for its construction. All chromosomes are **m** except the last smallest pair which is **sm**. The scale represents 10 μ m.

Table 2. Meiotic chromosome behaviour in different populations of *B. coridifolia*: diakinesis, no. of male individuals and cells analyzed; means: II_r = rod bivalent, II_R = ring bivalent and Qt = total chiasma number. In addition to the bivalents observed a quadrivalent was present in all cells

| Provenance and population no. | Diakinesis | | | |
|-------------------------------|----------------------|------------------------------|------------------------------|----------------------------|
| | (no. ind. and cells) | II_r X \pm SE (range) | II_R X \pm SE (range) | Qt X \pm SE (range) |
| Herrera (no. 6) | (4; 12) | 1.7 \pm 0.15 (1–2) | 5.3 \pm 0.15 (5–6) | 16.3 \pm 0.15 (16–17) |
| Junction 12 and 16 (no. 7) | (5; 13) | 0.15 \pm 0.08 (0–1) | 6.9 \pm 0.6 (6–7) | 17.9 \pm 0.08 (17–18) |
| Perdices (no. 8) | (4; 6) | 0.57 \pm 0.16 (0–1) | 6.43 \pm 0.16 (6–7) | 17.23 \pm 0.23 (16–18) |
| Tandil (no. 9) | (5; 7) | 1.57 \pm 0.19 (1–2) | 5.43 \pm 0.19 (5–6) | 16.4 \pm 0.19 (16–17) |

manent. We never observed a microspore mother cell with 9 bivalents in 663 cells that were studied (Table 1). The observed wide range of the translocation system suggests that it is very old and previous to the present wide distribution of the species. However, the possibility that it could have arisen more than once should not be discarded.

On the other hand, the few female plants that were studied were structural homozygotes and showed 9 bivalents in diakinesis.

A limited number of plant species of different families have reciprocal translocation as a characteristic component of their genetic system. The establishment of permanent heterozygosity for a reciprocal translocation is favored when chromosomes are of similar size, metacentric, and chiasmata are terminally located allowing an alternate orientation. The absence of interstitial chiasmata indicates restriction of crossing-over to the terminal segments. This adds to the restriction of recombination between the chromosomes involved in the translocation. As a consequence the translocated chromosomes are inherited together and behave like a single linkage group.

In *B. coridifolia* the four largest chromosomes that are involved in the sex-limited translocation system are

in fact metacentric of very similar size and have only terminal chiasmata. If there is alternate segregation, balanced gametes would be produced and different gene complexes carried by the translocated and the non-translocated chromosomes would segregate as separate units. With a few exceptions in all dioecious plants the sex chromosomes are the largest (GRANT 1999; AINSWORTH 2000).

In some dioecious plants permanent translocation heterozygosity has been produced, at least in the male sex, through the association of the translocations with the sex determination system and a translocation multivalent is characteristic of the heterozygous sex (BARLOW 1981). The mistletoe genus *Viscum* shows permanent sex-associated translocation heterozygosity in most of its dioecious species, distributed through Africa, Europe and Asia (WIENS and BARLOW 1973, 1975; WIENS and BARLOW 1979; BARLOW and WIENS 1975, 1976; BARLOW et al. 1978; BARLOW 1981; BARLOW and MARTIN 1984). In these species male plants have a multivalent at meiosis, ranging from 4 to 6, 8, 10 or 12 chromosomes. The translocated chromosomes function as a large Y chromosome.

In the plant kingdom dioecy results from the suppression of one sex in an hermaphrodite individual. About

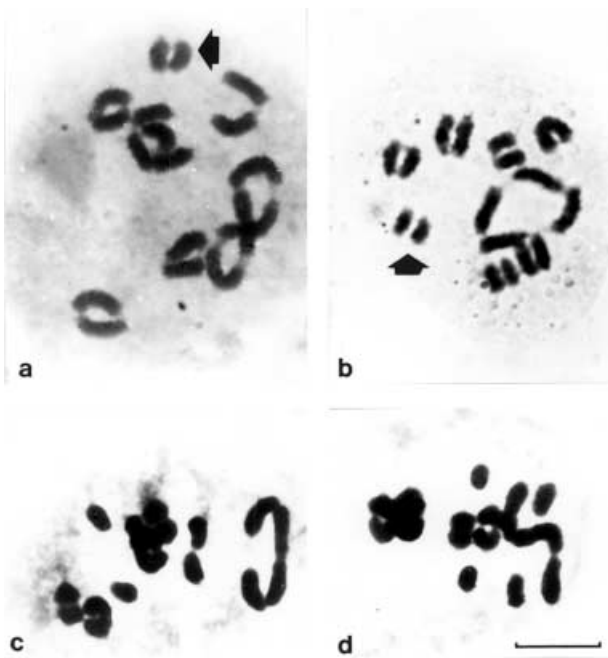


Fig. 3. Diakinesis and metaphase I cells of male plants of *B. coridifolia*. a–b = diakinesis. Arrow point at the smallest bivalent. c–d = metaphase I, in c the segregation is adjacent, in d is alternate; d shows a chain of 4. In all cases the quadrivalent appears to the right of the cell. The scale represents 10 μ m.

10% of the world flora (PROCTOR et al. 1996) and about 6% of the angiosperm species (RENNER and RICKLEFS 1995) are dioecious. This could result from mutations that affect different hormone systems concerned with flower development (BARLOW 1981). In mosses and higher plants the same hormones are involved in sexual differentiation. Cytokinins are feminizing, gibberellins and auxins are masculinizing

in *Mercurialis annua* (DURAND and DURAND 1990). In other genera the contrary is true (AINSWORTH 2000). It is likely that mutations causing maleness and femaleness would occur in different gene systems and would therefore be non-allelic. This possibility has been suggested by ROSS and WEIR (1976) and CHARLESWORTH and CHARLESWORTH (1978). The non-allelic genes for dioecy must be linked because if not genetic recombination could produce hermaphrodites, males, females and even sterile specimens. CHARLESWORTH and CHARLESWORTH (1978) have suggested that unlinked genes for dioecy would be selected against. In most dioecious species the genes for dioecy should occur on the same chromosome, with no recombination between the loci involved in sex determination.

Most of the South American species of *Baccharis* are diploids. In sixteen species that were studied by the senior author and associates the male plants always had 9 bivalents at diakinesis or metaphase I (ROZEMBLUM et al. 1985; HUNZIKER et al. 1989, 1990; WULFF et al. 1996). The only species so far known to consistently have quadrivalents in the males is *B. coridifolia*. Dioecy appears to be highly stable in its expression in *B. coridifolia* and other species and never monoecious plants are observed in all species except one, *B. monoica* (NESOM 1998), which is the only monoecious in a total of about 450 species of the genus.

We have to assume that: a) the *B. coridifolia* translocation system has evolved from the more common non translocation system existing in those species having 9 bivalents in male meiosis, b) the strictly male bivalent system determines also dioecy normally, c) the new translocation system must have

Table 3. Centromere orientation in quadrivalents of 12 male plants at metaphase I. (*Perdices* population)

| Plant no. | RINGS | | | CHAINS | | | Number of cells |
|-----------|-----------|----------|--------|-----------|----------|--------|-----------------|
| | Alternate | Adjacent | Linear | Alternate | Adjacent | Linear | |
| 1 | 2 | 2 | – | – | – | – | 4 |
| 2 | – | 1 | – | 1 | – | – | 2 |
| 3 | 5 | 1 | 2 | 2 | – | – | 10 |
| 4 | 3 | – | – | 2 | 1 | – | 6 |
| 5 | 2 | 2 | 1 | – | – | – | 5 |
| 6 | 23 | – | – | – | – | – | 23 |
| 7 | 14 | – | – | – | – | – | 14 |
| 8* | 12 | 3 | – | 1 | – | – | 16 |
| 9 | 22 | 1 | – | 1 | 1 | 1 | 26 |
| 10 | 2 | 1 | – | 1 | – | – | 4 |
| 11 | 16 | 1 | – | 1 | – | – | 18 |
| 12 | 4 | 1 | – | – | – | – | 5 |
| Total | 105 | 13 | 3 | 9 | 2 | 1 | 133 |
| % | 78.95 | 9.77 | 2.25 | 6.77 | 1.50 | 0.75 | |

* This plant had an extra floating quadrivalent of smaller size.

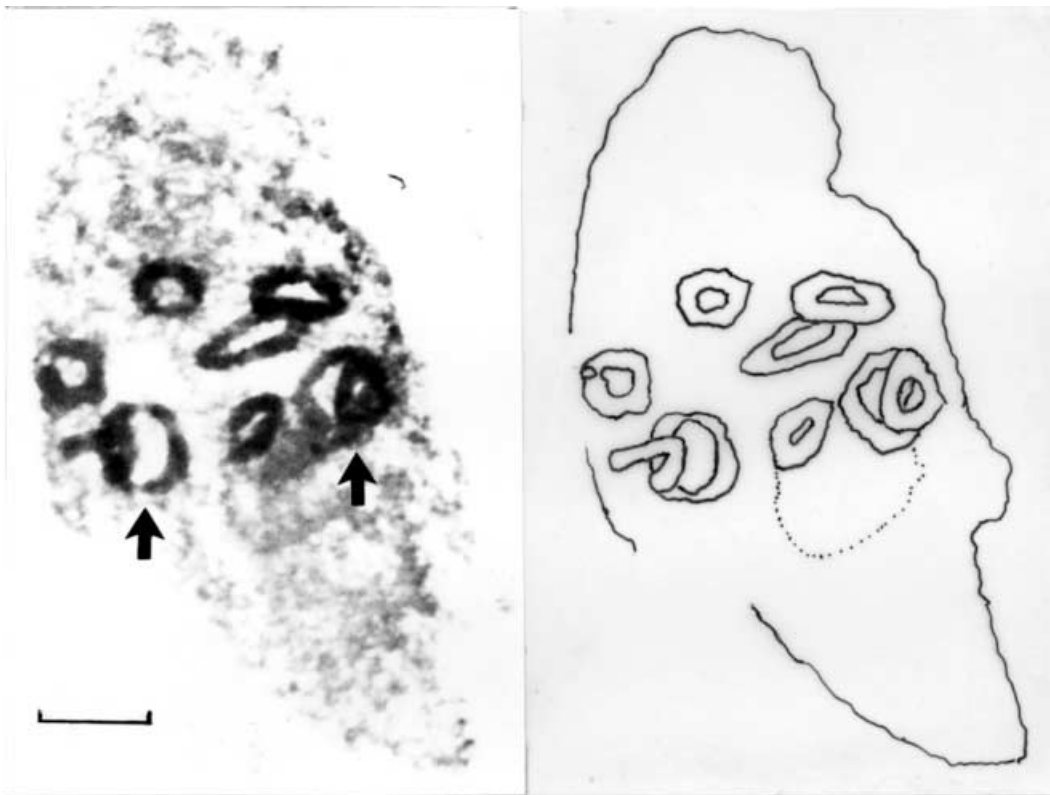


Fig. 4. Diakinesis in a megaspore mother cell showing 9 bivalents. Arrows point at the two largest non translocated bivalents. The scale represents 10 μm .

Table 4. *Sex ratios in Baccharis coridifolia*

| Locality | Male plants | Female plants | Sex ratio ♂/♀ | χ^2 expected 1:1 |
|-----------------|-------------|---------------|---------------|-----------------------|
| Tandil | 274 | 149 | 1.83 | 36.92* |
| Perdices (1999) | 61 | 19 | 3.21 | 22.05* |
| Perdices (2000) | 57 | 22 | 2.59 | 15.51* |

* $p < 0.001$.

conferred an advantage in order to become completely established through *B. coridifolia* range (Fig. 1). The model we have devised satisfies these three conditions. In the bivalent forming species one of these bivalents must be involved in sex determination with at least two loci. We may postulate the following genotypes for both types of species (forming a quadrivalent or not):

- Mm** = non functional ovary in male plants
- mm** = normal ovary in female plants
- Nn** = normal stamen in male plants
- nn** = absence of stamens in female plants.

The male plants would be heterogametic (**Mm Nn**) and the female plants homogametic (**mm nn**) (Fig. 5).

According to WODEHOUSE (1945) in *Baccharis* the flowers appear to be at least partly wind pollinated.

Very occasionally we have observed some insects that could participate in the pollination of *B. coridifolia*, such as *Chauliognathus scriptus* (Coleoptera, Cantharidae). Drs. G. Bernardello and L. Galetto (pers. comm.) have informed us that in the Córdoba province, Argentina have observed that *Thecla* sp (Lycaenidae, Lepidoptera) is a pollinator of *B. coridifolia*.

If insect pollination is not effective, on account of the small size of the inflorescences or due to occasional yearly reduction of the entomofauna, the action of the wind with an abundance of male plants would ensure pollination and reproduction. Male plants in higher frequency than females would be an advantage for the populations. This could be achieved through higher fitness of male plants. In fact, it appears that in general male-biased ratios in

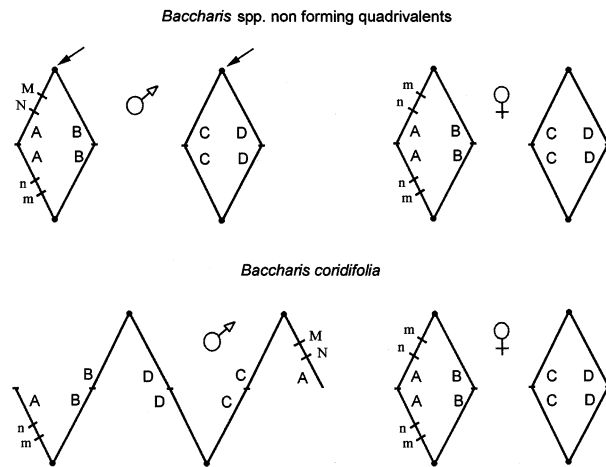


Fig. 5. Model for the origin of the sexual translocation system in *B. coridifolia* from species forming 9 bivalents. Possible breakage points for the reciprocal translocation are indicated by arrows. **M** and **n** are symbols for the postulated non-allelic factors for a dominant female-suppressor and a recessive stamen suppressor, respectively. The translocated chromosomes AC and BD occur only in male plants. Female plants would be structurally homozygous for the original AB and CD chromosomes and carry **mm nn** genotypes and form only bivalents. The quadrivalent is depicted as a chain for simplicity; when forming a ring both A arms are connected by a terminal chiasma.

dioecious plants would be more common than female-biased ratios. (LLOYD and WEBB 1977; MEAGHER 1981; ÅGREN 1988). If a translocation has occurred in the male of an ancestor species forming only 9 bivalents, with a breakage point near the centromere a quadrivalent would be formed and the translocated chromosomes would carry the male factors. A possible model is shown in Fig. 5 assuming non allelic sex determining factors.

In *B. coridifolia* important chemical differences between male and female plants have been reported. TOKARNIA and DOBEREINER (1975) reported that toxicity is increased 4 to 8 times after flowering and seed set. Male plants and vegetative plants have lower amounts (1/5 to 1/10) of macrocyclic trichotecenes whereas in the female plants that have undergone pollination the levels of these trichotecenes is much higher (JARVIS et al. 1991). When *B. coridifolia* female plants flower, the levels of the macrocyclics increase dramatically and are highest in the seeds, where concentration reaches extraordinary levels (0.3–5% by dry weight) in the seed coats (JARVIS et al. 1988).

In *Baccharis coridifolia* the situation is probably comparable to that of species of *Viscum*: the quadrivalent of the male plants being a sex multivalent. The male plants are structural and genic heterozygotes whereas the female ones are structural and genic

homozygotes (Fig. 5). The translocated segments probably carry, besides the staminate condition determining genes, some genes complexes that are responsible for the chemical differences between male and female plants and also some that promote heterotic effects on the male plants, which would have higher adaptive value. The selection pressure favoring male plants must be strong and as a result the sex ratios are highly skewed male predominant (Table 5). This excess of male plants, observed in several populations at different localities, probably ensures a good supply of pollen to compensate for the vagaries of insect pollination at flowering time. The advantage of male plants having a quadrivalent would be that now four chromosome arms can accumulate more heterotic alleles in the male plants. In the bivalent forming species this can occur only in two chromosome arms (Fig. 5). In the male plants the translocated chromosomes would function as a big sex bivalent, the male plants being permanently heterozygous for the interchange complexes.

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